Two papers dealing with F. Moewus' work on Chlamydomonas. Translated by E. M. Lederberg from German texts furnished by K. G. Grell at Woods Hole, Mass., August, 1954.

(These papers are in press in Zeitschr. Naturforschung)

Investigations on copulatory ability in Chlamydomonas eugametos. Herbert Förster & Lutz Wiese. Max. Planck Inst. Biol. (Hartmann Section, Tübingen).

The experiments of the work below should verify the frequently doubted contributions of Moewus with C. eugametos. The experiments were carried out with the greatest care, and I have myself in all cases been convinced that they always took place in the stated way and were always reproducible. Because the basic observations of Moewus could not be confirmed with the material made available, it was not possible to examine the relationship of the male and female gamones to light, and their quantitative alterations by light. It could only be proved that filtrates of male and female gametes contain differently acting hormones. Experiments with these filtrates will be reported later. Max Hartmann

***

In 1951, Dr. Moewus left behind with us a clone culture of both sexes and zygotes of C. eugametos var. simplex. We intended to reproduce his experiments and to confirm them. The study was carried out at the wish of Prof. Hartmann and Prof. Kuhn. Already with the fundamental experiments, we were confronted with disagreement. The essential experiments could not be tackled for that reason.

The clones were cultured on Knop agar (1.5%) at different light sources, especially with neon tubes HNW 40 (Osram) with a daily illumination period of 12 hours.

After flooding the agar plate with liquid (Knop solution 0.1%, 1% soil solution) (1:10 with the best distilled water) the cells were completely flagellated and motile not only in the light but also in the dark (1). No circumstances were found in which the cells remained non-motile after flooding. The cells were regularly fully motile within a half hour after flooding; in rare cases it lasted up to 2 hours. Previously during the cultivation on agar, a very small % of the cells (0-90% usually, at most 5%) were flagellated (2).

Moreover, nothing was altered after we tested 1-8 days placement in the dark. Precise causes for the diminution in percentage of flagellated cells could not as yet be determined. Affecting factors are age of the culture, consistency of the agar, and extent of the growth. Even cultures which beforehand contained no flagellated cells were completely motile in the dark. Motile cells remain motile and flagellated in liquid even after placement in the dark for an hour to a whole day (3).

While the experiments on motilization occurred consistently, highly variable behavior was found with regard to copulatory ability. We found that the cells after flooding in the dark could be likewise copulatory (4). If both sexes were mixed together and flooded in the dark one evening, often the next morning many copulating pairs were found; occasionally the agglutination reaction is initiated within a few seconds to several minutes after illumination. Insofar as the material is generally very reactive, long retardation periods do not occur. Reactivity was tested in parallel with experiments in the light. If one tests dark cultures of both sexes against reactive light cultures, it is shown that one sex (designated as female by Moewus) becomes nearly without exception fully able to copulate in the dark. On the other hand, dark males tested with reactive light females generally show a delay. Clump formation is initiated several seconds to a few minutes after mixing the sexes. If one illuminates such a dark culture of males, the retardation is decreased. The necessary illumination period for the instantaneous reaction (e.g. clump formation from 10 seconds after mixture of the sexes) is thereby at most greater than 2 minutes, perhaps 10-20 minutes.

It can consequently be confirmed that light influences copulatory ability, but the obligatory light-dependency and the quantitative relationships of Moewus were not reproduced (5). The influence of different kinds of light has not been investigated so far. Experiments with filtrates in

place of illumination and experiments with illuminated filtrates could not be cleanly carried out on account of the negative results in the dark which could not confirm that motile and non-copulatory cells were available. Experiments in this direction proceeded very variably and gave no unambiguous conclusion.

Motile cells unable to copulate ought also be obtained by completely different means, through the copulation-inhibiting reagent, rutin. According to Moewus, rutin inhibits the ability to copulate up to a dilution of 10^{-7}/l, thereby blocking gamone formation (6). The cultures, were, however, able to copulate in the presence of rutin supplements without exception. No differences were obtained from the distilled-water controls. We tested rutin made available by Prof. Kuhn, von Schuchardt, and Moewus in concentrations of 10^{-1} to 10^{-7} g/l on a series of different clones. Cultures from agar containing rutin were also unaffected.

Dr. Moewus had not furnished hermaphroditic strains of C. eugametos for different kinds of experiments (termones). Gones [zygote products] were isolated from zygote material. To begin with, tetrad analyses were superfluous for this purpose. The zygotes were easily germinated by the freezing method used by Moewus and the liberated gones immediately isolated and grown up as clonal cultures. No bisexuals (hermaphrodites) were found among 1094 analysed clones, the sex ratio amounting to 564 males: 530 females. According to Moewus, 3% hermaphrodites were obtained by crossing over.

An interpretation of the discrepancies with Moewus' investigations was not found. The studies on copulation phenomena will be continued.

Bibliography

F. MOEWUS

1) Arch. Protistenk. 80:469-526. p.475. 1933.

Jb. wiss. Bot. 86:753-783. p.757ff. 1938a.

Biol. Zbl. 60:597-626. p.617. 1940.

Erg. Enzymforsch. 12:173-206. p.174f. 1951.

2) 1938a s.755, 757.

3) " 758ff.

4) 1k9 1933. p.475 & 491f; 1951: 177f.

5) 1938a: 755f, 767f.

6) 1951: p.179f & 192f.

7) Ber. Dtsch. bot. Ges. 54:45-57. 1936.

Biol. Zbl. 58:516-536. 1938b.

II. Experiments on copulatory ability in Chlamydomonas eugametos.

The agglutinating effect of sex-specific gamones described by Moewus in 1933 was verified. The light-dependence for the release and activity of these gamones is described. From the viewpoint of new findings, the action of light-dependent gamones described later by Moewus is discussed.

The Agglutination-affecting Gamones.

Cells of C. eugametos cultured on agar are able to copulate if they are transferred to a liquid medium. If both sexes are brought together, an intensive clumping reaction ensues. The clumps separate after some time into isolated copulating pairs. If the suspensions of cells prepared for copulation are centrifuged or filtered, the cell-free supernate induced clumping in cells of the opposite sex which were able to copulate. The cells in copulatory condition thus release a sex-specific gamone into the culture fluid. This filtrate activity was described by Moewus in 1933. We could consistently reproduce it. In significant details, our findings differ from the account of Moewus.

1) According to Moewus the filtrates can only work if bacteria are present. We find, on the contrary, also maximal activity in bacteria-free cultures. The activity remained even in sterile filtrates passed through Jena glass filters. Filtrates from the latter simply showed a diminution of activity. Apparently the active components are adsorbed to the filter. Whereas the first ml which passed through the filter is often completely ineffective, optimal activity occurs after some time with a larger quantity of filtrate.

2) Moewus states that the active filtrates exhibit a lowering of the freezing point (FP) of about 10°. In our experiments the FP of the filtrates remains at 0° just as in the pure culture fluid. FP modifications are not present, at least not of this order of magnitude. The filtrates had optimal activity before and after freezing.

3) Moewus further states that the filtrates lost their activity after 12 hours. We found that the retention of the activity of the gamone is temperature-dependent. At room temperature ($19^{\circ} \pm 1^{\circ}$) the activity disappears in 2-4 days. It makes no difference if the filtrate is kept illuminated or not. In the icebox ($4^{\circ} \pm 1^{\circ}$) the activity is retained for several weeks. Higher temperatures destroy the activity even after a short time. The table (not given) shows the order of magnitude of the temperature-dependence. The persistence of activity is shorter in filtrates not optimally active.

The Light-dependence of the Action of the Gamone.

If male and female cultures are flooded in light, the cells become copulatory. Filtrates from the illuminated cultures cause typical clump formation with cells of the opposite sex. Both sexes thus release gamones in the light. Moreover, both sexes become reactive to the gamones in the light.

If the male and female cultures are flooded in the dark one evening, and the next morning are centrifuged free of cells in the dark, the supernate derived from the females is active, while on the other hand, that from the males is not. The filtrate from a male culture only becomes active after illumination.

The reactivity of the cells parallels that of the gamones. The females become fully reactive in the dark: with the addition of male filtrates they form clumps immediately. The dark male cells do not react to the female gamone: they become reactive only after illumination.

If female filtrates are added to reactive males, clump formation starts immediately. If the experiment is carried out in the light, the reaction begins to disappear in ca one hour and is entirely gone after 4-5 hours. If there is much filtrate for few cells, the reaction is

maintained longer. If there is little filtrate for many cells, the reaction disappears faster. The specified time is based on 1 ml filtrate which was delivered to 1 ml cells at a density of 10^6 cells/ml. The duration of the reaction of females to male filtrates is shorter. Under the same conditions it disappears fully after perhaps 2-3 hours. If one again adds supernatant at the end of the reaction it acts cell-specifically: the supernate of the females acts on males; the supernate of the males acts on females.

Illuminated, fully reactive cells placed in the dark after the addition of filtrate, show characteristic differences to the conditions of light according to sex. If one places female filtrates on males, the agglutination reaction deteriorates rapidly in the dark, i.e. the male cells quickly lose their reactivity to the female gamone in the dark, and the female gamone does not become saturated. The female gamone is still demonstrable in supernates prepared after a few hours. The supernate is still active on illuminated male cells. After the same interval, the female gamone is no longer demonstrable in the light experiment.

On the other hand, the agglutination reaction is carried out in the dark when male filtrates are placed on females. Compared with the light experiment it is only insignificantly weaker. The male gamones are used up in the dark. If supernates are prepared from the treated females after a few hours, they act specifically only on males, not on females.

DISCUSSION

In his work of 1933 Moewus described the action of agglutinating gamones. In his later work, he has investigated the light dependence of copulatory ability without making reference to this gamone reaction, and then replaced the effect of light by filtrates. With it he wished to demonstrate a second gamone effect. This gamone II effect is linked to

crocetindimethylester according to Moewus. In our experiments we had, above all, the purpose of reproducing this gamone II effect which stands as the central focus of Moewus' investigations. We came to disagree on that. Moewus attacked the problem of gamone II effect by adding the filtrates from copulatory cells of the same sex to non-copulatory dark cells. According to Moewus, under the influence of the filtrates, the cells became copulatory in the absence of light. The filtrate would thus contain substances which would be formed normally by the cells only in light, and which are decisive for copulatory ability. The substances were defined as sex-specific mixtures of cis and trans crocetindimethylester.

The filtrate induced copulatory ability of the dark cells was tested by the mixture of both sexes. In the belief that the gamone I effect (agglutinating action) is bound to the presence of bacteria, Moewus could ignore the occurrence of gamone I effect in these tests when he worked with bacteria-free cultures. On the basis of our results that bacteria-free cultures are also active, a test on gamone II effect is at least greatly impeded because gamone I effect is always noted. If one gives, for instance, active male filtrates to unreactive male dark cells, and then tests these dark males against the reactive females, clump formation occurs of necessity, solely because of gamone I effect, for in the females an isoagglutination is developed as a result of male filtrates. It is thus difficult to demonstrate a gamone II effect in the test of clump formation, for gamone I effect always interferes, and the appearance of a gamone II effect can not be distinguished.

It does not appear possible to test gamone II effect at the next copulation state either. Pair formation appears only by the mixture of both sexes. Therefore, just for the agglutination alone, 4 factors are necessary (all essential also for the complete progress of the copulation):

gamone formation in the females, gamone formation in the males, reactibility of the females to gamone I, reactibility of the males to gamone I. Two of these factors are light-dependent and thus conceal a second light-dependent gamone effect. Our year-long experiments on the influence of light on copulation leave no room for a second light-dependent gamone effect. They can be fully explained by the light-dependence of gamone I effect. The females need no light to develop copulatory capacity; light is a significant factor for the copulatory capacity of the males. It is true that this light-dependence is not obligatory, as fundamentally, copulations can take place in the dark. If the mixture of cells of both sexes are left for a longer time, clumps and pairs appear even in the dark. It is still not clear which factors hereby are introduced in the place of the light. The course of copulation is at least inhibited for several hours in the dark, however, in contrast to the controls placed in the light.

Especially informative are the last described findings on the light-dark experiments on males. They remain in clear contradiction to the filtrate experiments of Moewus. In the light, males brought to full reactivity lose their reactivity to the female gamone after transfer to the dark.

In its support is, in addition, the pair formation in cultures which become fully copulatory in the light, and then when mixed in the dark, were strongly retarded, in contrast to the controls. Occasionally it [copulation] ceases entirely. Gamones I are readily released in light, and they continue to be actively produced in the dark. According to Moewus' experiments in which filtrates should substitute for light, gamone II must also remain active in the dark. Nevertheless, copulatory capacity is very quickly lost. Even if the effect is not absolute, it is still a strong factor. The latter effect leads to nothing further on the behavior of the male gamone I in contrast to that of the female. Of

course if such males which become reactive in the light and then placed in the dark, are tested against the reactive light females, clump formation occurs. The male gamone I is produced and acts on the females. During observation under the binocular microscope the male cells were illuminated and consequently after a short interval, became reactive again.

The carrier of agglutinating gamone effect is precipitated by ammonium sulfate, and is not dialyzable.

We see for our part no possibility that the effect of gamone II of Moewus is demonstrable. Further determinations are in progress.